Domesticating variegated thistle

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Abstract

Variegated thistle (*Silybum marianum* (L.) Gaertn.) is grown overseas for its seed, which contain silymarin, a liver detoxicant. The plant is naturalized in New Zealand where it is a noxious weed. The development time, yield and silymarin content of the naturalized New Zealand line was compared to those of a German line at four sowing dates.

The New Zealand line was slower maturing than the German line but had a much higher silymarin content, averaging 2 % compared to 0.6 %. Relative yields of the two lines were highly dependent on sowing date. In a second trial, yields and silymarin content of the German line, with or without plant growth regulator, no N fertiliser or 100 kg N/ha, and thinned or unthinned plant populations, were compared over 2 years. Thistle height was significantly reduced using a growth regulator, and yield was increased in one year. Fertiliser N increased yield in both years. There was no significant effect of agronomic treatment on silymarin content.

***Additional Key Words*:** milk thistle, silymarin, nitrogen, density, plant growth regulator, silybin, cultivar.

Introduction

Variegated thistle (*Silybum marianum* (L.) Gaertn.), also known as Milk Thistle, Holy Thistle, Blessed Thistle, Lady's Thistle, and St. Mary's Thistle, is a native herb from southern Europe that is an important weed in temperate areas of the world, including New Zealand where it is classified as a noxious weed. However, it is grown commercially in some countries for the seed ((Martin *et al.*, 2000). The seed contains 1.5-3 % silymarin, which is used widely in Germany and other countries for treatment of hepatitis, cirrhosis and other diseases of the liver (Morazzoni and Bombardelli, 1995; Flora *et al.*, 1998). Silymarin is a mixture of flavanolignans, the main active constituent being silybin (Morazzoni and Bombardelli, 1995). The pharmacology of silymarin and silybin is well documented (Flora *et al.*, 1998; Fraschini *et al.*, 2002).

The crop has been trialled at Crop and Food Research since 1994 to establish its potential as a commercial seed crop in New Zealand. Earlier trials with plants grown from seed harvested from wild plants in New Zealand showed that New Zealand variegated thistle has a biennial growth habit (Martin *et al.*, 2000). In Europe, variegated thistle is grown commercially as an annual crop (Hecht *et al.*, 1992; Schunke, 1992), and is drilled and headed like a cereal crop.
Seed of a German line was obtained and included in two trials with the aim of:

- comparing the effect of time of sowing on seed yield of New Zealand and German variegated thistle in a small-scale trial.
- determining the effect of agronomic treatments on seed yield of German variegated thistle grown as a semi commercial crop.

Materials and Methods

Three trials were undertaken, a Time of Sowing trial from 1999 to 2000, and two Agronomy trials, one in 1999-2000, and other in 2000-2001. The trials were carried out at Crop and Food Research's Lincoln farm. The soil is a deep (>1.6 m) Templeton sandy loam (*Udic Ustochrept*, USDA Soil Taxonomy) (New Zealand Soil Bureau 1968) with an available water holding capacity of c. 190 mm/m of depth. Soil physical properties on an adjacent site were detailed by Martin *et al.*, (1992). The Time of Sowing trial and first Agronomy trial followed a long term pasture, and followed ulluco in the second Agronomy trial. Soil nutrient status (0-15 cm) under both trials established in 1999 was pH 6.6, Ca 11 (MAF Quick Test), P 10 µg/ml, K 6 (MAF QT), S 2 ppm, Mg 14 (MAF QT), Na 7 (MAF QT), NO_3-N 9 ppm and total N 0.20 %. In the trial established in 2000, soil nutrient status was pH 6.7, Ca 10, P 8 ug/ml, K 5, S 2 ppm, Mg 15, Na 7, NO_3-N 11ppm and total N 0.14 %.

Time of sowing trial

Seed of a German line of variegated thistle seed was sourced from Germany. Seed for the New Zealand line was sourced from a previous year's trial (Martin *et al.,* 2000), which was initially gathered from wild populations on Banks Peninsula. Both seed lines had a laboratory germination of 80 %. The trial was set out using a split plot design with sowing dates as the main plots and cultivars as the sub-plots. There were four replicates, with the main plots laid out in randomized blocks.

The four sowing dates for the trial were 13 October 1999, 17 December 1999, 18 February 2000 and 11 April 2000. Initial seedbed preparation for each planting was by ploughing and rota-crumbling, with final cultivations by hand hoeing and raking. Each sowing was in 3 m by 3 m plots with 30 cm rows and 15 cm between plants in the row. The seeds were sown by hand about 2 cm deep in the rows opened by hoe.

The plots were hand-weeded. All the plots were watered by watering can after sowing to ensure good establishment. No fertiliser was applied. Measurements of emergence and growth stages were made weekly.

The middle six rows of the plot were harvested when about 50 % of the seed heads appeared to be mature. Plant numbers, plant heights and plot fresh weights were measured. A weighed five plant subsample was taken for counts of buds, flowers and seed heads. It was then divided into seed and vegetative material, which were both dried at 35°C, and then weighed. A 50 g subsample of seed was taken for seed weight and silymarin determination.

Agronomy trials

The two agronomy trials were very similar. They were drilled on 20 October 1999 and 20 October 2000, respectively, using an Oyjord drill at a rate of 20 kg seed/ha, designed to give a plant population of 80 plants/m^2 at 100 % establishment. The treatments in both were:

- no plant growth regulator (PGR), or 60 g/10 l of solution/50 m^2 of B-nine®(a.i. 850 g/kg daminozide) sprayed on using a knapsack sprayer at the start of stem elongation (10 January 2000 and 4 January 2001).
- no nitrogen (N) fertiliser, or top-dressed with 100 kg N/ha as urea at the start of bud formation (11 January 2000 and 16 January 2001).
- plant population (PP) thinned to approximately 13 plants/m^2 or plant population as planted (27 plants/m^2) on 15 December 1999 or 15 December 2000.

The combination of these factors gave eight treatments (2x2x2 factorial)

The 1999-2000 trial had a split plot design, with PGR as main plots and N and PP as a 2 x 2 factorial set of subplots with the main plots laid out in a randomised block design. The 2000-01 trial was laid out as a randomized block design for the eight treatments. Both trials had four replicates, with a plot size of 7 m x 1.5 m. Both trials were hand weeded twice in late November and early December, and were irrigated using sprinklers immediately after the nitrogen was applied.

The trials were harvested on 15 March 2000 and 6 March 2001 when around 90 % of the heads had matured. Immediately before harvest, the height of three plants taken at random was measured in each plot. A 7.35 m^2 area of each plot was harvested with a Hege plot harvester with the drum removed. The material was collected from the back of the harvester in wool sacks and was allowed to dry indoors for 2 weeks before being threshed through the Hege harvester and the seed collected in the normal way. The remainder of the plants were weighed and a 300-1500 g subsample dried in a forced draught oven. The seed was sieved then dried at 35°C for two nights, and 50 g subsamples taken for silymarin analysis and seed weight determination.

Silymarin analysis

For each trial, the samples from pairs of replicates were combined into one sample for silymarin analysis. The seeds were ground, and a subsample extracted in a continuous infusion soxhlet apparatus for two hours. An aliquot of the extract was taken and evaporated to dryness under a stream of nitrogen. Samples were then dissolved in 1 ml of 1:1 methanol:water for HPLC analysis. Analysis was on a Phenomenex Prodigy ODS column (15cm x 4.6 mm I.D.) held at 35°C. The solvent was 44:56 methanol:water with a flow of 1 ml/min. UV detection was at 210 nm, with confirmation at 287 nm. Component concentrations were determined with reference to a standard containing silybin A and silybin B, each at about 50 µg/ml. Other components (silychristin, silydianin, isosilybin) were tentatively assigned by retention time, and their concentrations determined by peak area relative to the silybin standard. Total silymarin content was derived from the sum of all component concentrations. The results were analysed by analysis of variance using the GenStat statistical package (Genstat Committee, 2000)

Results and Discussion

Time of sowing trial

The German line matured significantly ($P<0.05$) faster than the New Zealand line (Table 1), but the difference between the two lines varied from 9 days for the April sowing through 18-52 days for the February and October sowings, respectively, to 158 days for the December sowing. The German line sown in December produced seed by late April, whereas the New Zealand line did not produce seed until late September.

There was a wide variation in seed yields, ranging from 21 to 496 g/m^2 (Table 1). The seed yield from the October-sown German plots was considerably reduced due to bird damage before the crop was harvested. All the other treatments were harvested earlier to try to reduce the problem of birds eating the seeds before harvest.

Highest yields were from the February-sown New Zealand line. The New Zealand line has adapted to a biennial habit (Martin *et al.,* 2000) and in the wild, seed germinates in late summer, producing a plant that remains vegetative through the winter. It then becomes reproductive and produces seed in late December. The high seed yields are a reflection of the significantly higher number of mature heads for the February sowing. In contrast, the German line has been selected for sowing in spring and harvesting in autumn (Schunke, 1992), although the results from this trial indicate that it could be sown in autumn, and still produce good seed yields.

The seed yield of the December-sown New Zealand line was very low, probably because flower pollination and seed set occurred in cold wet weather during the winter (Martin *et al.,* 2000).

Yields of the New Zealand line increased with increasing numbers of seed heads, and, with the exception of the October sowing, the German line appeared to follow the same trend. There was little difference in mature seed head number between the two lines for the October and December sowings, but mature seed head numbers were considerably higher in the New Zealand line compared to the German line for the February sowings.

Table 1. Date of first mature seed head, with days from sowing in brackets, number of mature seed heads, buds and flowers, seed yield, seed weight, harvest index and silymarin content of German (G) and New Zealand (NZ) lines of variegated thistle sown on four dates.

	Date of 1st mature seed head (days from sowing)		Mature seed heads (number/m^2)		Bud and flower (number/m^2)		Seed yield (g/m^2)		Seed weight (mg)		Harvest index (%)		Silymarin content (%)	
Sowing date	G	NZ	G	NZ	G	NZ	G	NZ	G	NZ	G	NZ	G	NZ
Oct 1999	2 Feb (112)	14 Mar (152)	192	191	31	215	101	235	22.6	20.9	4.2	4.4	0.63	1.75
Dec 1999	17 Apr (121)	22 Sep (279)	100	109	140	311	221	21	22.8	12.0	7.8	0.4	0.80	2.23
Feb 2000	14 Nov (269)	14 Dec (299)	137	352	216	289	171	426	15.5	16.6	3.9	11.0	0.37	1.73
Apr 2000	11 Dec (244)	20 Dec (253)	181	228	146	145	392	298	19.3	18.2	13.0	11.3	0.75	2.23
LSD[1]	(8.1)		116.2		43.0		152.4		1.60		2.06		0.671	
LSD[2]	(8.4)		135.7		43.6		157.5		1.65		2.34		0.445	

[1] LSD: least significant difference between two cultivars for the same sowing date at the 5 % level (df = 9, obtained from the results of ANOVA).

[2] LSD: least significant difference between two cultivars for the same sowing date at the 5 % level (df = 11, obtained from the results of ANOVA).

Except for the April sowing, the New Zealand line had significantly more buds and flowers at harvest than the German line, a probable reflection of a more indeterminant habit associated with its biennial life cycle. Selection work to increase the number of heads maturing together would increase seed yield in headed crops.

The harvest index (seed dry weight as a proportion of total crop dry weight) was very low in this trial (maximum 13 %). Despite its shorter life cycle, the German line did not have a higher harvest index. Further selection work is required to reduce the prickly vegetative mass and increase the seed yield to make harvesting of this crop easier.

The major finding of this trial was the three- to fourfold higher silymarin content of the New Zealand line compared to the German line. Chiavari *et al.,* (1991), found up to a twofold difference in silymarin content between lines from different countries. In our results, the early maturing line had a lower seed silymarin content, whereas Chiavari *et al.,* (1991) found that an earlier maturing line had double the silymarin content of a later maturing line. Reports of silymarin contents vary widely in the literature, with 0.65-1.46 % reported from Egypt (Omer *et al.,* 1993; Hammouda *et al.,* 1993), up to 3.42 % in Germany (Schunke, 1992) and 2.43 % to 4.29 % in Italy (Chiavari *et al.,* 1991). Future work should therefore concentrate on the New Zealand line rather than the German line, as the higher seed silymarin content will be more acceptable to processors.

There were also significant differences in silymarin content between sowing dates for the German line, suggesting that environmental conditions may affect silymarin synthesis and accumulation in the seed.

Agronomy trials

Seed yields were much lower in these two trials (mean 28 g/m^2 in 1999-2000 and 75 g/m^2 in 2000-2001) compared to the time of sowing trial. In overseas work, German headed trials averaged 115 g/m2 (Schunke, 1992). Yields were reduced in the agronomy trials because the crop had to be allowed to dry down to make it fit for heading, giving birds the opportunity to consume a significant amount of seed. Bird control may be necessary if birds are still a significant problem with larger scale commercial crops.

The plant growth regulator, B-nine, reduced height from 1.6 m to 1.3 m ($P<0.05$), which suggests a simple and practical method of making the crop easier to manage and harvest. It also increased seed yield by 19 % in one year ($P<0.05$), suggesting a significant change in allocation of assimilate. Further work on growth regulators on this crop would therefore seem warranted.

Application of N fertiliser increased seed yield by 273 % and 18 % ($P<0.05$) in the two trials but had no significant effect on silymarin content or crop height. In Germany, high rates of N fertiliser reduced seed yields, but had no effect on silymarin content (Schunke, 1992). In Egypt, increasing N fertiliser application also increased variegated seed yields, but not silymarin content (Omer *et al.,* 1993; Hammouda *et al.,* 1993). Omer (1996) found that ammonium sulphate produced higher seed yields and silymarin content than urea or ammonium nitrate, indicating that the nitrogen-sulphur balance may be important for variegated seed yield and quality.

Table 2. Height, seed yield, seed weight, and silymarin content at two rates of plant growth regulator, two rates of nitrogen fertiliser, and two plant populations on a German line of variegated thistle.

	Height (m)		Seed yield (g/m^2)		Seed weight (mg)		Silymarin content (%)	
Growth reg.	1999-2000	2000-1	1999-2000	2000-1	1999-2000	2000-1	1999-2000	2000-1
None	1.58	1.52	26.5	77.3	24.6	23.3	1.01	1.45.
B-nine	1.31	1.29	28.8	92.1	25.1	23.2	0.90	1.37
LSD (5 %)[1]	0.118	0.046	7.08	11.29	0.89	0.76	1.35	0.200
Fertiliser								
Nil	1.43	1.37	14.8	77.7	24.5	23.7	0.85	1.39
100 kg N/ha	1.45	1.43	40.54	91.7	25.1	22.8	1.06	1.42
LSD (5 %)[2]	0.095	0.046	6.03	11.29	0.36	0.76	0.37	0.200
Population								
Thinned	1.39	1.40	29.7	79.9	25.0	23.4	0.92	1.35
Unthinned	1.49	1.41	25.7	89.5	24.7	23.1	0.98	1.47
LSD (5 %)[2]	0.095	0.046	6.03	11.29	0.36	0.76	0.37	0.200

[1] 1999-2000 d.f.=3, 2000-1 d.f.=18, except for silymarin where 1999-2000 d.f.=1, 2000-1 d.f.=7

[2] 1999-2000 d.f.=18, 2000-1 d.f.=19 (because of two missing plots), except for silymarin where 1999-2000 d.f.=6, 2000-1 d.f.=7

Density had no effect on crop height, seed yield or silymarin content. In Egypt, increasing the row spacing of variegated thistle decreased seed yield but increased silymarin content. However, the actual seeding rate or plant populations were not reported (Omer *et al.,* 1993). Our results here, and observations of other trials, suggest that the crop tends to be self-regulating, producing a certain number of seed heads per unit area irrespective of the number of plants.

There were no significant interactions between growth regulator, nitrogen and plant density on crop height, seed yield, seed weight or silymarin content.

The lack of any significant response in silymarin content to agronomic treatments (Table 2) may be indicative of the fact that seed yields are such a small part of total yield (Table 1). The agronomy trials demonstrated that the crop can be successfully drilled and mechanically harvested. Future agronomic research can therefore concentrate on inputs and practices to increase harvest index and harvestable seed yield.

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